

CLAIMS

What is claimed is:

- 5 1. A method for preparing a stem cell comprising:
 - A) obtaining an aspirate from liposuction;
 - B) subjecting the aspirate from liposuction to centrifugation to obtain a cell fraction
 - C) subjecting the cell fraction to centrifugation
 - 10 by specific gravity; and
 - D) collecting a cell layer with lower specific gravity than that of erythrocytes.
- 15 2. The method according to Claim 1, wherein said aspirate from liposuction is prepared using saline or Ringer's solution.
- 20 3. The method according to Claim 1, wherein said centrifugation is conducted at a speed of a range equal to or less than 800 x g.
- 25 4. The method according to Claim 1, wherein said centrifugation is conducted at a speed of a range equal to or less than 400 x g.
5. The method according to Claim 1, wherein said centrifugation by specific gravity is conducted at a speed of a range between 370 x g and 1,100 x g.
- 30 6. The method according to Claim 1, wherein said centrifugation by specific gravity is conducted using medium which has a specific gravity of 1.076 to 1.078 g/ml at 20 degree Celsius.

7. The method according to Claim 1, wherein the medium of said centrifugation by specific gravity is selected from the group consisting of Ficoll, Percoll and sucrose.

8. The method according to Claim 7, wherein the medium of said centrifugation by specific gravity is Ficoll.

9. The method according to Claim 1, wherein the specific gravity of the collected cell layer is at a range of between 1.050 and 1.075.

10. The method according to Claim 1, wherein the collection of said cell layer is conducted using a pipette.

11. The method according to Claim 1, further comprising the step of culturing said cell layer in a medium containing components selected from the group consisting of DMEM, M199, MEM, HBSS, Ham's F12, BME, RPMI1640, MCDB104, MCDB153(KGM) and a mixture thereof.

12. The method according to Claim 1, wherein the centrifugation by specific gravity comprises density gradient centrifugation.

13. The method according to Claim 1, further comprising the step of removing blood cells.

14. A method for preparing a stem cell comprising:
A) obtaining material from liposuction; and

B) subjecting the material from liposuction to centrifugation to obtain a cell fraction without isolation of fat tissue.

- 5 15. The method according to Claim 14, further comprising the step of subjecting the material to a condition where at least a portion of cells are separated from the material.
- 10 16. The method according to Claim 15, wherein the condition is for degradation of extracellular matrices.
17. The method according to Claim 15, said degradation of extracellular matrices is achieved by a collagenase.
- 15 18. The method according to Claim 14, further comprising the step of removing supernatant in step B).
19. The method according to Claim 14, further comprising the step of filtering the material from the step B).
- 20 20. The method according to Claim 14, further comprising the step of removing blood cells.
- 25 21. The method according to Claim 14 wherein the step of removing blood cells comprises adding a component of degrading blood cells.
- 30 22. A method for preparing a stem cell comprising:
i) obtaining material from liposuction;
ii) subjecting the material to a condition where at least a portion of cells are separated from the

material, without isolation of fat tissue;

iii) subjecting the material to centrifugation;

iv) adding a component degrading blood cells to the material and agitating the material;

5 v) subjecting the material to centrifugation to obtain a pellet; and

vi) aspirating supernatant of the material from the pellet.

10 23. The method according to Claim 22, wherein the step of subjecting the material to said condition comprises maintaining an aspirate from the liposuction.

15 24. The method according to Claim 22, wherein said material from liposuction comprises an aspirate from liposuction and fat.

20 25. The method according to Claim 22, wherein said condition in said step ii) comprises adding a collagenase.

25 26. The method according to Claim 22, wherein the centrifugation in said step iii) is conducted at 400-1200 x g.

27. The method according to Claim 22, wherein said component degrading blood cells comprises ammonium chloride and potassium bicarbonate.

30 28. The method according to Claim 27, wherein said ammonium chloride is comprised in the component at 155mM

29. The method according to Claim 27, wherein said potassium bicarbonate is comprised in the component at 10mM.

5 30. The method according to Claim 22, wherein said centrifugation in said step v) is conducted at 400-1200 x g.

31. The method according to Claim 22, wherein said
10 pellet contains a stem cell.

32. A stem cell prepared by the method according to any of Claims 1-31.

15 33. The stem cell according to Claim 32, which expresses at least one protein selected from the group consisting of CD13, CD29, CD34, CD36, CD44, CD49d, CD54, CD58, CD71, CD73, CD90, CD105, CD106, CD151 and SH3.

20 34. The stem cell according to Claim 33, which expresses CD13, CD29, CD34, CD36, CD44, CD49d, CD54, CD58, CD71, CD73, CD90, CD105, CD106, CD151 and SH3.

25 35. The stem cell according to Claim 33, further expressing at least one protein selected from the group consisting of CD31, CD45, CD117 and CD146.

30 36. The stem cell according to Claim 32, which does not express CD56.

37. The stem cell according to Claim 32, which does not express at least one protein selected from the

group consisting of CD3, CD4, CD14, CD15, CD16, CD19, CD33, CD38, CD56, CD61, CD62e, CD62p, CD69, CD104, CD135 and CD144.

- 5 38. The stem cell according to Claim 37, which does not express CD3, CD4, CD14, CD15, CD16, CD19, CD33, CD38, CD56, CD61, CD62e, CD62p, CD69, CD104, CD135 and CD144.
- 10 39. The stem cell according to Claim 32, which expresses CD49d and does not express CD56.
40. A system for preparing a stem cell comprising:
- 15 A) means for obtaining an aspirate from liposuction;
- B) means for subjecting the aspirate from liposuction to centrifugation to obtain a cell fraction; and
- 20 C) means for subjecting the cell fraction to centrifugation by specific gravity.
41. The system according to Claim 40, wherein the system further comprises:
- 25 D) means for collecting a cell layer with lower specific gravity than that of erythrocytes.
42. A system for preparing a stem cell comprising:
- A) means for obtaining material from liposuction; and
- 30 B) means for subjecting the material from liposuction to centrifugation to obtain a cell fraction without isolation of fat tissue.

43. A system for preparing a stem cell comprising:

- i) means for obtaining material from liposuction;
- ii) means for subjecting the material to a condition where at least a portion of cells are separated from the material, without isolation of fat tissue;
- iii) means for subjecting the material to centrifugation;
- iv) a component degrading blood cells to the material and agitating the material;
- v) means for subjecting the material to centrifugation to obtain a pellet; and
- vi) means for aspirating supernatant of the material from the pellet.

44. A method for obtaining an explant comprising:

- A) obtaining an aspirate from liposuction;
- B) subjecting the aspirate from liposuction to centrifugation to obtain a cell fraction;
- C) subjecting the cell fraction to centrifugation by specific gravity;
- D) collecting a cell layer with lower specific gravity than that of erythrocytes;
- E) culturing the collected cell layer to obtain an explant.

45. A method for preparing a tissue transplant comprising:

- A) obtaining an aspirate from liposuction;
- B) subjecting the aspirate from liposuction to centrifugation to obtain a cell fraction; and
- C) culturing the collected cell layer to obtain a tissue transplant.

46. A method for preparing tissue transplant comprising:

- A) obtaining an aspirate from liposuction;
- 5 B) subjecting the aspirate from liposuction to centrifugation to obtain a cell fraction;
- C) subjecting the cell fraction to centrifugation by specific gravity;
- D) collecting a cell layer with lower specific
- 10 gravity than that of erythrocytes;
- E) culturing the collected cell layer to obtain a tissue transplant.

47. A method for transplanting a tissue transplant comprising:

- A) obtaining an aspirate from liposuction;
- B) subjecting the aspirate from liposuction to centrifugation to obtain a cell fraction;
- C) subjecting the cell fraction to centrifugation
- 20 by specific gravity;
- D) collecting a cell layer with lower specific gravity than that of erythrocytes;
- E) culturing the collected cell layer to obtain a tissue transplant; and
- 25 F) transplanting the tissue transplant.

48. Use of an aspirate of liposuction in preparing stem cells.

30 49. A method for preparing cells selected from the group consisting vascular endothelial precursor cells, adipocytes, cartilage cells, bone cells and muscle cells comprising the step of culturing a stem cell

obtained by the method according to any one of Claims 1-31.

50. A method for preparing a differentiated cell
5 comprising:

A) obtaining a mixture by mixing

a) the stem cell obtained according to any one of Claims 1-31, and

10 b) a differentiated cell corresponding to a desired site; and

B) culturing the mixture under sufficient conditions which allow the adipose-derived precursor cell to differentiate.

15 51. The method according to claim 50, wherein the differentiated cell is a mesenchymal cell.

52. The method according to claim 50, wherein the differentiated cell is selected from the group
20 consisting of adipocytes, bone marrow cells, osteoblasts, chondrocytes, fibroblasts, myofibroblasts, nerve cells, skeletal muscle cells, cardiac muscle cells, vascular endothelial cells, vascular smooth muscle cells, hepatic cells, renal cells, and pancreas
25 cells.

53. The method according to claim 50, further comprising providing an agent for promoting differentiation into said differentiated cell.

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54. The method according to claim 50, wherein the mixture is cultured in a medium containing at least one ingredient selected from the group consisting of

adrenocortical steroids, insulin, glucose, indomethacin, isobutyl-methylxanthine (IBMX), ascorbic acid and a derivative thereof, glycerophosphate, estrogen and a derivative thereof, progesterone and a derivative thereof, androgen and a derivative thereof, growth factors, pituitary gland extracts, pineal body extracts, retinoic acid, vitamin D, thyroid hormone, fetal bovine serum, equine serum, human serum, heparin, sodium hydrogen carbonate, HEPES, albumin, transferrin, selenates, linoleic acid, 3-isobutyl-1-methylxanthine, demethylating agent, histone deacetylating agents, activin, cytokine, hexamethylenebisacetamide (HMBA), dimethylacetamide (DMA), dibutyl cAMP (dbcAMP), dimethylsulfoxide (DMSO), iododeoxyuridine (IdU), hydroxyurea (HU), cytosine arabinoside (AraC), mitomycin C (MMC), sodium butyrate (NaBu), polybrene, and selenium.

55. A cell mixture, comprising a stem cell obtained according to any one of Claims 1-31; and a differentiated cell corresponding to a desired site.

56. The cell mixture according to Claim 55, wherein the cell mixture is subjected to conditions sufficient for inducing the differentiation of the stem cell.

57. A composition for cell transplantation comprising:
a) a stem cell obtained according to any one of Claims 1-31; and
b) a differentiated cell corresponding to a desired site.

58. The composition according to Claim 57, wherein the

transplantation is conducted at the desired site.

59. The composition according to Claim 57, wherein the ratio between the stem cell and the differentiated cell
5 is about 1:10 to about 10:1.

60. The composition according to Claim 57, wherein the ratio between the stem cell and the differentiated cell is about 1:2 to about 2:1.
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61. The composition according to Claim 57, wherein the ratio between the stem cell and the differentiated cell is substantially the same.

15 62. The composition according to Claim 57, wherein said differentiated cell comprises a mesenchymal cell.

63. The composition according to Claim 57, wherein the differentiated cell is selected from the group
20 consisting of adipocytes, bone marrow cells, osteoblasts, chondrocytes, fibroblasts, myofibroblasts, nerve cells, skeletal muscle cells, cardiac muscle cells, vascular endothelial cells, vascular smooth muscle cells, hepatic cells, renal cells, and pancreas
25 cells.

64. The composition according to Claim 57, further comprising at least one ingredient selected from the group consisting of adrenocortical steroids, insulin,
30 glucose, indomethacin, isobutyl-methylxanthine (IBMX), ascorbic acid and a derivative thereof, glycerophosphate, estrogen and a derivative thereof, progesterone and a derivative thereof, androgen and a

derivative thereof, growth factors, pituitary gland extracts, pineal body extracts, retinoic acid, vitamin D, thyroid hormone, fetal bovine serum, equine serum, human serum, heparin, sodium hydrogen carbonate, HEPES, 5 albumin, transferrin, selenates, linoleic acid, 3-isobutyl-1-methylxanthine, demethylating agent, histone deacetylating agents, activin, cytokine, hexamethylenebisacetamide (HMBA), dimethylacetamide(DMA), dibutyl cAMP (dbcAMP), 10 dimethylsulfoxide (DMSO), iododeoxyuridine (IdU), hyroxyurea (HU), cytosine arabinoside (AraC), mitomycin C (MMC), sodium butyrate (NaBu), polybrene, and selenium.

15 65. The composition according to Claim 57, wherein the stem cell and the differentiated cell are allogeneic to each other.

20 66. The composition according to Claim 57, wherein the stem cell and the differentiated cell are syngeneic to each other.

25 67. A method for treating or preventing a disease, disorder or abnormal condition associated with a failure of a differentiated cell, comprising:

A) providing a composition comprising:

a) a stem cell obtained according to any one of Claims 1-31; and

30 b) a differentiated cell corresponding to a desired site; and

B) administering the composition to a subject.

68. A medicament for treatment or prevention of a

disease, a disorder or an abnormal condition attributed to the deficiency of a differentiated cell, comprising:

a) a stem cell obtained according to any one of Claims 1-31;

5 b) a differentiated cell corresponding to a desired site; and

c) a pharmaceutically acceptable carrier.

69. Use of a mixture of: a) a stem cell obtained
10 according to any one of Claims 1-31; and b) a differentiated cell corresponding to a desired site, for preparation of a medicament for treatment or prevention of a disease, a disorder or an abnormal condition attributed to the deficiency of a
15 differentiated cell.

70. A method for treatment or improvement of a cosmetic condition, comprising the steps of:

A) providing a composition comprising:

20 a) a stem cell obtained according to any one of Claims 1-26; and

b) a differentiated cell corresponding to a desired site; and

B) administering the composition to a subject.

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71. A medicament for treatment or improvement of a cosmetic condition, comprising:

a) a stem cell obtained according to any one of Claims 1-31;

30 b) a differentiated cell corresponding to a desired site; and

c) a pharmaceutically acceptable carrier.

72. Use of a mixture of: a) a stem cell obtained according to any one of Claims 1-31; and b) a differentiated cell corresponding to a desired site, for preparation of a medicament for treatment or
5 improvement of a cosmetic condition.